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GENETIC DIVERSITY IN OVERWINTERED AND NON-OVERWINTERED *IPS PINI* (SAY) (COLEOPTERA: SCOLYTIDAE) IN IDAHO

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Abstract.—The pine engraver, *Ips pini* (Say), has two generations per year in ponderosa pine (*Pinus ponderosa* Lawson) in Idaho, one that develops in pine slash in the spring and one that develops in live trees in the summer. The summer generation overwinters to produce the spring generation the following year. Non-overwintered and overwintered beetles were sampled from two sites. Average heterozygosity was significantly higher in overwintered beetles in both groups. While the proportion of homozygous individuals did not differ significantly between overwintered and non-overwintered beetles from either site, the proportion of heterozygous individuals was significantly greater after overwintering in beetles from one of the two sites. The increase in genetic diversity after overwintering is consistent with observations that heterozygosity is favored by severe environmental conditions.

Key Words.—Insecta, Scolytidae, genetics, heterozygosity, stress

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The pine engraver, *Ips pini* (Say), is a widely distributed bark beetle infesting several species of pine in coniferous forests throughout North America. In Idaho, ponderosa pine (*Pinus ponderosa* Lawson) is most commonly infested. Typically, two generations of *I. pini* are produced each year in Idaho, one in spring and one in summer (Fig. 1). The F1 progeny of overwintered adults emerge in late spring and infest either fresh slash or live, standing trees that are typically immature, in dense stands, and moisture stressed. Like other bark beetle species, the pine engraver can mass attack live trees and kill them by feeding on the phloem, which disrupts the trees' food supply, and introducing pathogenic fungi. They may also infest tops of more mature trees, including those previously attacked by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, or the western pine beetle, *D. brevicomis* LeConte. The second generation (F2, or summer generation) matures in late summer and overwinters as adults in the litter beneath trees killed in the summer, or under the bark of these trees, most often at their base (Livingston 1979). Overwintered adults emerge in April and May and fly to infest fresh slash (recently felled trees and branches) or the tops of trees broken off by wind or snow. At that time, live trees are generally not attacked.

Ips pini exhibits the high levels of heterozygosity (average frequency of heterozygous individuals per locus) that characterize bark beetles in general. Average heterozygosity for 17 bark beetles—10 *Dendroctonus* species (Bentz & Stock 1986) and seven *Ips* species (Cane et al. 1990)—was 16.7 percent. *Ips pini* was 15.9 percent heterozygous (Cane et al. 1990).

The advantage of heterozygosity is frequently expressed as higher survival of

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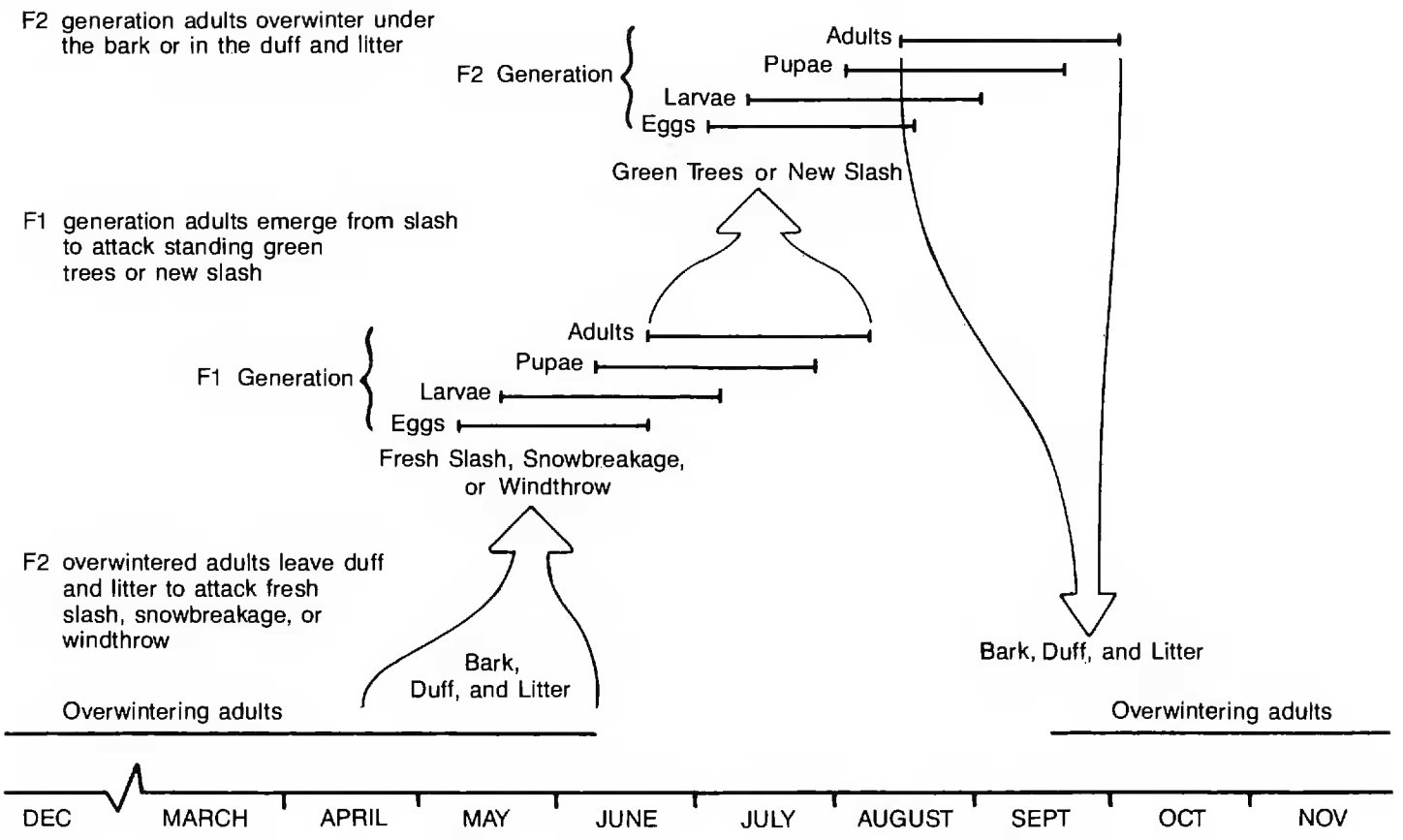

Figure 1. *Ips pini* life history in Idaho (modified from Livingston 1979).

heterozygous individuals under severe or stressful environmental conditions, even when these conditions are transient or episodic (e.g., Bryant 1974, 1976; Milkman 1978; Smith et al. 1975; Parsons 1971, 1987). Samollow & Soule (1983), for example, discovered a striking case of superior heterozygote survivorship among toads during the winter. Development of the mountain pine beetle in stressful environmental conditions—dry or thin phloem in the laboratory or in high-density infestations in the field—has been associated with increased levels of genetic diversity, measured as average heterozygosity (Stock & Amman 1983, Amman and Stock in press).

The high levels of inherent genetic diversity in *I. pini* populations, combined with differences in the severity of environmental conditions affecting a population over the course of a year, make the pine engraver an ideal subject for further studies of the relationship between heterozygosity and environmental stress. To initiate studies of this type, we measured heterozygosity in non-overwintered and overwintered F2 *I. pini* from two sites in northern Idaho.

Materials and Methods

Collections.—F2 beetles were obtained in August 1985 from standing ponderosa pine trees at Moscow Mountain (Latah Co., Idaho) and Greer (Clearwater Co., Idaho, approximately 80 km from Moscow Mtn.). Fifteen to 20 logs, approximately 1.5 m long and averaging 20 cm in diameter, were collected from each site.

Half of the logs from the Greer and Moscow sites were brought indoors in the fall, and beetles were collected as they emerged and flew to an illuminated white sheet. These beetles represented the F2 generation before overwintering. The other half of the logs from Greer were kept over the winter in the outdoor cage containing a 10 cm layer of soil and litter, and emerging beetles (overwintered F2s) were

Table 1. Enzyme characteristics, allele frequencies, heterozygosity (h), and average heterozygosity (H) in samples of *Ips pini* taken from two sites. F2 = summer generation (non-overwintered) and OW = overwintered F2s. Asterisks mark loci where observed genotype frequencies differed from expected Hardy–Weinberg values.

Locus	Anodal or cathodal[a]	Monomeric or dimeric[b]	RM[c]		Moscow Mountain F2	Moscow Mountain OW	Greer F2	Greer OW
AAT	A	D		*n*	(144)	(150)	(89)	(128)
			.45	p(1)	.05	.08	.04	.07
			.38	p(2)	.89	.84	.92	.88
			.31	p(3)	.06	.07	.04	.05
			.24	p(4)	—	—	—	—
				h	(.202)	(.280)	(.150)	(.218)
ADH	C	D		*n*	(150)	(150) **	(145)	(148)
			.27	p(1)	.07	.05	.07	.06
			.22	p(2)	.91	.83	.92	.82
			.17	p(3)	.01	.12	.01	.11
			.12	p(4)	—	—	—	.01
				h	(.167)	(.294)	(.149)	(.312)
AK	A	M		*n*	(78)	(149)	(113)	(150)
			.76	p(1)	—	—	—	.01
			.69	p(2)	.24	.29	.22	.24
			.61	p(3)	.74	.70	.76	.72
			.51	p(4)	.02	.01	.01	.02
			.40	p(5)	—	—	.01	.02
				h	(.394)	(.426)	(.374)	(.423)
CAT	A	D		*n*	(147)	(108)	(155)	(150)
			.26	p(1)	.02	.02	.01	.04
			.21	p(2)	.96	.91	.93	.91
			.16	p(3)	.02	.07	.05	.06
				h	(.078)	(.167)	(.132)	(.167)
G6PDH	A	M		*n*	150	149	148	146
			.20	p(1)	1.0	1.0	1.0	1.0
				h	(0)	(0)	(0)	(0)
GAPDH	C	D		*n*	(150)	(150) **	(155)	(148) **
			.27	p(1)	.06	.05	.06	.05
			.22	p(2)	.93	.84	.94	.78
			.17	p(3)	.01	.11	—	.16
			.12	p(4)	—	—	—	.01
				h	(.131)	(.280)	(.113)	(.363)
GUS	A	D		*n*	(149)	(111)	(132)	(150)
			.38	p(1)	.01	.03	—	.04
			.32	p(2)	.99	.92	.99	.93
			.26	p(3)	—	.05	—	.03
				h	(.020)	(.150)	(.020)	(.133)
IDH1	A	D		*n*	(150)	(150)	(158)	(150)
			.42	p(1)	—	—	—	—
			.36	p(2)	.89	.87	.89	.84
			.28	p(3)	.08	.09	.11	.11

Table 1. Continued.

Locus	Anodal or cathodal[a]	Monomeric or dimeric[b]	RM[c]		Moscow Mountain F2	Moscow Mountain OW	Greer F2	Greer OW
			.22	p(4)	.02	.04	.01	.05
				h	(.201)	(.233)	(.196)	(.280)
IDH2	C	M		*n*	(150)	(150)	(155)	(150)
			.16	p(1)	1.0	1.0	1.0	1.0
				h	(0)	(0)	(0)	(0)
LAP	A	M		*n*	(148)	(150)	(158)	(150)
			.34	p(1)	.02	.05	—	.03
			.28	p(2)	.98	.95	1.0	.97
				h	(.039)	(.095)	(0)	(.058)
LDH	C	D		*n*	(150)	(150) **	(154)	(148) **
			.27	p(1)	.06	.05	.06	.05
			.22	p(2)	.93	.84	.93	.78
			.17	p(3)	.01	.11	.01	.16
			.12	p(4)	—	—	—	.01
				h	(.131)	(.280)	(.131)	(.363)
MDH1	A	D		*n*	(150)	(150)	(156)	(150)
			.42	p(1)	.01	—	—	—
			.34	p(2)	.01	.01	—	.01
			.26	p(3)	.96	.97	.96	.96
			.18	p(4)	—	—	.02	.02
			.10	p(5)	.02	.01	.01	—
				h	(.078)	(.059)	(.078)	(.078)
MDH2	C	D		*n*	(150)	(150)	(158)	(150)
			.33	p(1)	—	—-	—	—
			.25	p(2)	1.0	1.0	.99	.98
			.17	p(3)	—	—	.01	.01
				h	(0)	(0)	(.020)	(.039)
ME	A	D		*n*	(150)	(150)	(158)	(150) **
			.31	p(1)	.01	.03	—	.03
			.26	p(2)	.98	.96	.99	.97
			.21	p(3)	—	.01	.01	—
				h	(.039)	(.077)	(.020)	(.058)
MPI	A	M		*n*	(150)	(149)	(77)	(150)
			.71	p(1)	.93	.89	.94	.93
			.66	p(2)	.07	.09	.05	.05
			.56	p(3)	–	.02	.01	.02
				h	(.130)	(.200)	(.114)	(.132)
PEP1	A	D		*n*	(150)	(150)	(158)	(150)
			.44	p(1)	—	–	.01	–
			.40	p(2)	.99	1.0	.99	.99
			.36	p(3)	.01	—	—	—
				h	(.020)	(0)	(.020)	(.020)
PEP2	A	D		*n*	(148) **	(146) *	(149)	(150)
			.27	p(1)	.01	.01	.02	.02

Table 1. Continued.

Locus	Anodal or cathodal[a]	Monomeric or dimeric[b]	RM[c]		Moscow Mountain F2	Moscow Mountain OW	Greer F2	Greer OW
			.21	p(2)	.47	.45	.54	.50
			.15	p(3)	.15	.52	.43	.45
			.09	p(4)	.35	.02	.02	.02
			.03	p(5)	.01	—	—	—
				h	(.634)	(.527)	(.523)	(.547)
PGI	A	D		*n*	(150)	(150)	(158)	(150)
			.37	p(1)	.01	—	.01	.01
			.32	p(2)	.74	.77	.68	.74
			.27	p(3)	.24	.22	.31	.24
			.24	p(4)	.01	.01	—	.01
				h	(.394)	(.358)	(.441)	(.395)
SOD	A	M		*n*	(150)	(150)	(156)	(150)
			.27	p(1)	1.0	1.0	1.0	1.0
				h	(0)	(0)	(0)	(0)
				H (%)	14.0	18.3	13.1	18.9

[a] Direction of migration during electrophoresis.
[b] Molecular structure.
[c] Relative mobility.

collected in spring 1986. The other half of the logs from Moscow Mtn. were left on site over the winter and brought indoors in the spring for collection of beetles as they emerged and flew to an illuminated white sheet. Voucher specimens of male and female beetles from each location were placed in the William F. Barr Entomological Museum, University of Idaho.

Genetic Analysis.—The genetic makeup of populations was estimated using data obtained by horizontal starch gel electrophoresis. Approximately 150 overwintered and non-overwintered beetles from each site were analyzed. Techniques and stains used followed those of Higby & Stock (1982) and Bentz & Stock (1986).

Analyses of data were performed using BIOSYS-1 (Swofford & Selander 1981) and SAS (SAS Institute 1988). Initially, genotype frequencies from male and female beetles in each group were compared using a contingency chi-square test. Where no significant differences occurred, data on males and females were pooled for further analysis. Observed genotype frequencies were compared to values derived from random-mating (Hardy-Weinberg) expectations using a chi-square test.

Levels of heterozygosity were compared using two different approaches. In the first, Nei's (1975) average heterozygosity (H) was calculated and compared with two-tailed *t*-tests on transformed data to identify differences between non-overwintered and overwintered beetles from the two sites. In the second approach, the proportion of heterozygotes, taken by direct count, was compared using a procedure for categorical data modeling.

Results

Nineteen loci from 16 enzyme systems were assayed. Of these, three loci (G6PDH, IDH2, and SOD) were monomorphic in all samples and 16 loci (AAT, ADH,

Table 2. Percent of heterozygous individuals at 16 polymorphic loci in non-overwintered (F2) and overwintered (OW) *Ips pini* from two sites.

Locus	Moscow Mountain		Greer	
	F2	OW	F2	OW
AAT	16.7	19.8	10.1	23.4
ADH	14.7	13.3	15.2	23.0
AK	41.0	47.0	43.4	33.3
CAT	8.3	12.2	9.7	13.3
GAPDH	12.7	12.7	4.5	25.7
GUS	1.8	7.4	1.5	11.3
IDH1	16.0	21.3	17.7	22.0
LAP	1.3	6.7	0	4.0
LDH	13.3	12.7	13.0	24.3
MDH1	6.0	4.0	5.8	7.3
MDH2	0	1.3	1.3	2.0
ME	0.7	0.6	0	0
MPI	12.7	16.8	9.1	14.7
PEP1	0	0	1.3	1.3
PEP2	52.7	37.7	48.3	46.7
PGI	46.0	42.7	47.5	37.3
Average % heterozygotes per locus	15.2	16.0	14.3	18.1

AK, CAT, GAPDH, GUS, IDH1, LAP, LDH, MDH1, MDH2, ME, MPI, PEP1, PEP2, and PGI) were polymorphic in one or more samples. No significant differences occurred between genotype frequencies of male and female beetles from different locations within overwintered and non-overwintered groups. Therefore, the males and females were pooled for comparison of the genetic makeup of overwintered and non-overwintered beetles from the two sites (Table 1).

Deviations from expected genotype frequencies occurred not at all in non-overwintered F2s from Greer and only once (PEP2) in non-overwintered F2s from Moscow Mtn.. Deviations from Hardy-Weinberg expectations were observed in overwintered F2s from Moscow Mtn. (at four loci: ADH, GAPDH, LDH, and PEP2) and in overwintered F2s from Greer (three loci: GAPDH, LDH, and ME). At both sites, average heterozygosity (H) was significantly higher in the overwintered beetles than in non-overwintered beetles: 18.3 vs. 14.0% ($P < 0.05$) in the Moscow Mtn. beetles and 18.9 vs. 13.1% ($P < 0.01$) in Greer beetles.

The proportion of homozygotes taken by direct count (Table 2) did not differ between overwintered and non-overwintered beetles from either site. However, the proportion of heterozygotes taken by direct count was significantly higher ($P < 0.01$) in overwintered than in non-overwintered beetles from Greer (18.1 vs. 14.3%). Although there was also a slightly larger proportion of heterozygotes in overwintered beetles from Moscow Mtn. (16.0 vs. 15.2%), this difference was not significant.

Discussion

The classical view of genetic diversity in nature assumes that the fittest form of virtually every locus is the homozygous form. However, electrophoretic studies have revealed a large amount of genetic diversity within natural populations (e.g.,

Selander 1976, Ferguson 1980), and have shown that heterozygous individuals are more adaptable than their more homozygous counterparts. In a number of plant and animal species, relatively heterozygous individuals display superior growth and survival (e.g., Garton et al. 1984, Mitton & Grant 1980, Mitton & Koehn 1975, Soule 1980), and under laboratory conditions, heterozygous populations are often able to maintain larger population sizes or biomass than less heterozygous populations (Beardmore 1983).

The precise biochemical basis of heterozygote superiority is unknown, but it has been suggested that the presence of multiple forms of a gene product in heterozygous individuals confers relatively greater flexibility and latitude of a biochemical process. These advantageous effects can, in most cases, be attributed to heterozygosity per se, not to the effects of specific gene combinations (Mitton & Grant 1984). Heterozygosity appears to broaden the range of physiological tolerance and function relative to homozygosity (Mitton & Grant 1984, Smith et al. 1975). Similarly, a genetically diverse population is considered more adaptable to changing environmental conditions and more likely to survive over long time periods.

A central difference between non-overwintered and overwintered populations of *Ips pini* is the severe environmental conditions to which the latter group is exposed. Our primary question concerned the effect of overwintering on the level of heterozygosity in the F2 generation of *Ips pini.* Overwintering significantly increased levels of average heterozygosity in beetles from both sites that were studied, and the proportion of heterozygous individuals was significantly greater after overwintering at one of the two sites. Thus, this study lends some support to the hypothesis that severe environmental conditions tend to select for a more heterozygous population.

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